ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Biological Sciences 3 (4): 195-201, 2010
ISSN 1996-3351

Antimicrobial Resistance Patterns of *E. coli* Detected from Hospitalized Urine Culture Samples

[1]Mohsen Mohammadi, [2]Ehsan Ghasemi, [3]Hamid Mokhayeri,
[3]Yadollah Pournia and [3]Hadis Boroun
[1]Department of Microbiology,
Lorestan University of Medical Sciences, Khoram Abad, Iran
[2]Department of Microbiology, Ilam University of Medical Sciences, Ilam, Iran
[3]Lorestan University of Medical Sciences, Khoram Abad, Iran

Abstract: This study evaluated urine cultures and the antimicrobial resistance patterns of *E. coli* detected from urine culture. This study was performed on 520 hospitalized urine samples including 401 females and 119 males during one year (2008-2009) in Khorramabad City (West Iran). The urine cultures and antibiotic sensitivity tests based on Kirby-Bauer method were analyzed according to the antibiotic resistance pattern of each bacterium. A total of 520 urine samples were analyzed out of which 129 (24.8%) showed significant growth and 115 (89.1%) were gram-negative bacteria. The most frequently detected gram-negative bacterium was *E. coli* with 85 cases (73.9%). Urinary Tract Infection (UTI) was seen in 79.1% of the females as compared to 20.9% of the males (p<000.1). The most resistance rates for *E. coli* detected from urine culture were to ampicillin with 98.4% and to amoxicillin with 83.7%, respectively. The most antibiotic sensitivity rates were to amikacin with 93.3%, ciprofloxacin with 91.5%, nitrofurantoin with 89.8% and to nalidixic acid with (78.7%), respectively. Present findings demonstrated the significance of resistance increase of *E. coli* detected from urine culture to various groups of antibiotic drugs, caused by the irregular use of antibiotics.

Key words: UTI, drug resistance, negative-gram

INTRODUCTION

Urinary Tract Infections (UTI) one of the most common disease encountered in medical practice today (Nakhjavani *et al*., 2007). Urinary tract infection is one of the most common bacterial infections and gram-negative bacteria are among the most prevalent bacteria detected from UTI patients, particularly females. *Escherichia coli* is the most causative in this regard known as the most prevalent UTI pathogen, followed by other gram-negative bacteria of Enterobacteriaceae (Selvarangan *et al*., 2004; David and Williams, 1996; Sydney, 1990). More than 80% of urinary tract infections occur in outpatients and *E. coli* accounts for more than 50% of the infections in these patients (Blomgran *et al*., 2004; Jha and B apat, 2005). These infections are important due to antibiotic resistance, recognizing their major pathogens, resistance patterns and their antibiotic sensitivity.Consequently, drug resistance complicates urinary infections treatment and this

Corresponding Author: Ehsan Ghasemi, Department of Microbiology,
Ilam University of Medical Sciences, P.O. Box 69391-77143, Ilam, Iran

fact necessitates the appropriate use of drugs, along with the invention and synthesis of new drugs (Hasan *et al*., 2007). Hospital UTIs often happen in hospitals in patients with interior urinary catheter and their resistance to drugs is an increasing global problem particularly in developing countries (Laupland *et al*., 2005). The prevalence rates of antibiotic resistance in bacteria detected from hospital infections differ in terms of bacterial species, hospital conditions and even with regard to countries and may be associated with regional epidemics (Bajaj *et al*., 1999; Gupta *et al*., 2001). The aim of the present study was to investigate urine cultures concerning the frequency of gram-negative bacteria detected from cultured urine samples and to evaluate the antibiotic resistance patterns of the most frequent gram-negative bacterium detected from urine culture in acquired urinary tract infections of hospitalized patients. Therefore, investigating the antibiotic resistance patterns of *E. coli* seem to be essential concerning the increasing use of antibiotics followed by increasing antibiotic resistance and diversity of *E. coli* sensitivity in various parts of the world. The study aimed to determine the prevalence rate and the common antibiotic resistance patterns of *E. coli* detected from urinary infections.

MATERIALS AND METHODS

This study was carried out to evaluate the antimicrobial resistance patterns of *E. coli* detected from urine culture on 520 hospitalized urine samples in several hospital, including 401 females (77.1%) and 119 males (22.8%) in terms of types of pathogens and microbial sensitivity patterns in Khorramabad city (West Iran) during one year (2008 and 2009). Urine samples were aseptically collected in sterile containers and then cultured on blood agar and McConkey agar, using a calibrated loop, delivering 0.01 mL of the sample. Then, culture medium was incubated at 37°C overnight, The bacterial growth of >105 colony forming units cfu mL^{-1} of urine was considered as positive culture while the growth of <104 cfu mL^{-1} of infected urine was regarded as the nonexistence of pathogen growth and negative culture. After the positive samples were processed using biochemical tests for isolation and identification of microorganisms, according to standard procedure. The standard disk diffusion microbial sensitivity test based on Kirby-Bauer method on Muller-Hinton agar was utilized for all the isolates to assess the antibiotic resistance using antibiotic disks on *E. coli* as the most frequent detected gram-negative bacterium After incubation at 35°C for 24 h, zone size was measured. The collected data were processed statistically using the SPSS 15 software and chi-square tests. Fisher's exact test was also applied considering a 5% p-value.

RESULTS AND DISCUSSION

Out of all the 520 urine culture samples, 129 cases including 106 females (82.1%) and 23 males (17.8%) were positive and had remarkable growth. Consequently, a significant difference was found between gender and infection rate ($p<0.001$). The frequencies for the pathogens in the positive cases were as follows: 115 cases (89.1%) were gram-negative bacteria and the most frequent gram-negative bacterium was *E. coli* with 85 cases (73.9%) and other bacteria included Klebsiella with 17 cases (14.7%), Proteus with 13 (11.3), *Pseudomonas* with 11 (9.5%), *Enterobacter* with 6 (5.2%), *Citrobacter* with 2 (1.7%), respectively and other gram-negative bacteria with 5 cases (4.3%) (Table 1). The resistance rates of *E. coli* detected from urine culture was found to be 98.4% to ampicillin and 83.7% to

Table 1: The frequency of gram-negative bacteria separated from positive urine culture

Organism	Frequency	
	Number	Percentage
E. coli	85	73.9
Klebsiella	17	14.7
Proteus	13	11.3
Pseudomonas	11	9.5
Enterobacter	6	5.2
Citrobacter	2	1.7
Other gram-negative bacteria	5	4.3

Table 2: The resistance and sensitivity rates of separated *E. coli* to antibiotics in antibiogramic test

Antibiotics	Resistance (%)	Antibiotics	Sensitivity (%)
Ampicillin	98.4	Amikacin	93.3
Amoxicillin	83.7	Ciprofloxacin	91.5
Amoxicillin-clavulanic acid	69.3	Nitrofurantoin	89.8
Trimethoprim	56.7	Nalidixic acid	78.7
Cephalexin	48.1	Gentamicin	68.9
		Ceftazidime	67.2

amoxicillin, 69.3% to amoxicillin-clavulanic acid, 56.7% to trimethoprimand, 48.1% to cephalexin, respectively. Additionally, the most sensitivity rates were reported for amikacin with 93.3%, ciprofloxacin with 91.5%, nitrofurantoin with 89.8%, nalidixic acid with 78.7%, gentamicin with 68.9% and ceftazidime with 67.2% (Table 2).

This study investigated 520 clinical urine samples of hospitalized patients and their culture in several hospitals in Khorramabad (West Iran). It was revealed that firstly the most frequent bacteria detected from urinary tract infections were gram-negative bacteria (89.1%) and secondly *E. coli* (73.9%) was the most frequent detected gram-negative bacterium and it shows a similarity with the results of other researches (Astal, 2005; Jha and Bapat, 2005; Andrade *et al*., 2006; Kresken and Hafner, 2006). Urinary tract infection is a common relapsing disease which infects approximately 11 million women yearly and is considered as one of the major causes of hospital infections (Foxman and Riley, 2001). In the present study, a significant difference was found between the infection rates in males and females showing a similarity with the results of other studies in this regard (Kurutepe *et al*., 2005; Zilevica, 2005). The prevalence of antibiotic resistance of the urinary tract infection microorganisms is increasing worldwide and is an important factor in appropriate antibiotics choosing and taking them as treatments. The *E. coli*, among the most frequent detected organisms, shows various resistance rates to diverse antibiotics and in present study, the most resistance rates of *E. coli* detected from urine culture were found to ampicillin with 98.4% and to amoxicillin with 83.7% while the least resistance rates and the most sensitivity were, respectively reported for amikacin with 93.3%, ciprofloxacin with 91.5%, nitrofurantoin with 89.8% and nalidixic acid with 78.7%. Therefore, the following comparisons can be done between the results of the present study and the compatible results of other studies in Iran and in other countries.

Studies Carried Out in Iran

In a study carried out on 213 urine samples in Tabriz-West Iran, by Ghotaslou *et al.* (2005) cases (79%) of *E. coli* were isolated so that the most resistance was to ampicillin with 95% while the least resistance rates were to nitrofurantoin with 26.7%, nalidixic acid with 22.5%, amikacin with 18.3% and to ciprofloxacin with 4.3%.

In another study performed in Ghazvin-West Iran, on 363 positive urine culture samples, firstly, the infection rates for females and males were reported as 71.2 and 28.7%,

respectively; secondly, *E. coli* was isolated in 75.6% of the cases; and thirdly, its most resistance rates were, respectively reported to ampicillin, cotrimoxazole and cephalothin while the least rates were, respectively found for ceftazidime, nalidixic acid, ciprofloxacin, nitrofurantoin, amikacin and gentamicin (Vaezzadeh and Sharifi-Yazdi, 2001).The results of a study by Fallah *et al.* (2008) revealed that the most prevalent separated bacterium in urinary tract infection was *E. coli* (75%) and its most resistance was to amoxicillin (70.4%) while, the least resistance was to amikacin (3.7%).

Borji *et al.* (2001) results included the most resistance rates of 91.05% to ampicillin and 80.48% to cotrimoxazole versus the least resistance rates of 2.44% to ceftriaxone and 35.9% to ciprofloxacin.

Mokhtarian *et al.* (2006) found *E. coli* as the most frequent infection agent in a study in Gonabad-East Iran, on 353 positive urine samples of *E. coli*. In their study, the urinary infection rate in females (78.5%) was significantly more than the rate in males (21.5%). The resistance rates of 100 and 99.7% were found to amoxicillin and ampicillin, respectively. However, the sensitivity rates were 85% to ciprofloxacin and 60.1% to ceftizoxime (Mokhtarian *et al.*, 2006).

Studies Carried Out Abroad of Iran

Kader *et al.* (2004) conducted a research on 1764 urine samples in Saudi Arabia and separated *E. coli* from 58% of the cases. Nearly 50% of the separated *E. coli* showed resistance to amoxicillin, clavulanic acid and trimethoprim.

Jha and Bapat (2005) introduced *E. coli* as the most frequent bacterium separated from urine samples (50%) in a study carried out in Japan on 244 urine samples. Additionally, urinary infection was found to be more prevalent in women than in men and the most and the least resistance rates were reported for ciprofloxacin and nitrofurantoin, respectively.

In Hasan *et al.* (2007) study conducted in India on 2436 positive urine samples, 50.7% of *E. coli* were isolated and the urinary tract infection of 70.5% for women was reported compared with the infection rate of 29.5% for men. Moreover, the resistance rates of *E. coli* to gentamicin, ciprofloxacin and amikacin were reported to be 90, 68 and 33%, respectively (Hasan *et al.*, 2007).

In Astal's study in the Gaza Strip on 49 urine samples, *E. coli* was isolated from 52.5% of the samples and the most resistance to amoxicillin (78.7%) and the least to nitrofurantoin (2.7%) were demonstrated (Astal, 2005).

In a survey by Tankhiwale *et al.* (2004) on *E. coli*, the most resistance rates were found to cotrimoxazole (82%) and ampicillin (79.9%), while the most sensitivity rates were to nitrofurantoin (62%) and ceftizoxime (58.7%).

The results of Inabo and Obanibi (2006) study on *E. coli* isolated from urinary infections in Nigeria demonstrated the least resistance to ciprofloxacin and the most resistance to cotrimoxazole and nalidixic acid.

In Tamberkar *et al.* (2006) study performed on 68 urinary samples in India, *E. coli* was separated from 59% of the cases and the most resistance was demonstrated to ampicillin (87%) and cotrimoxazole (91%), while the most sensitivity was found to nitrofurantoin (71%).

Bartoloni *et al.* (2006) in a study on *E. coli* reported the highest resistance to ampicillin (85%), trimethoprim and sulfametoxazol (84%) and the lowest resistance to ceftriaxone (0.1%) and amikacin (0.2%).

Based on the results of the present study and many studies in this regard, some of which were mentioned above, it is concluded that *E. coli* is the most important and prevalent

bacterium isolated from urinary infections and its resistance to some drugs including ampicillin and amoxicillin, etc., exists continuously in most parts of the world, while its resistance rates to some other drugs including nalidixic acid, gentamicin, etc., vary and are increasing showing the fact that different species of *E. coli* play roles in causing urinary infections. It also may be attributed to other factors including the prevalence of some species of *E. coli* epidemically in some parts of the world, in addition to other factors such as age, gender, individual hygiene, hospital infections, irregular and inappropriate use of antibiotics without antibiograms, or inappropriate and non-standard disks utilized in laboratories. Moreover, currently the resistance to some drugs such as amikacin, ciprofloxacin, nitrofurantoin, imipenem, etc., is fortunately low; however, we should be aware of the increasing resistance to these drugs in the future. For instance, although fluoroquinolones are among the most effective drugs in treating urinary drugs (Kurutepe *et al*., 2005), diverse studies have revealed the increasing resistance of the bacterium to fluoroquinolones. For example, in a study carried out by Nakhjavani *et al.* (2007) in Tehran on the resistance of *E. coli* in urinary tract infections to fluoroquinolones, the resistance rates of 49.3% to nalidixic acid, 44.5% to ofloxacin, 41.4% to norfloxacin and 40.2% to ciprofloxacin were reported (Nakhjavani *et al*., 2007). Other studies were also in this realm (Alos *et al*., 2005; Rebeecah *et al*., 2006; Seputiene *et al*., 2006). The results of other various studies revealed that the resistance of *E. coli* to ciprofloxacin, the most effective drug to urinary tract infections, is increasing. In Astal's study in China during the years of 1998 to 2000, the resistance increased from 46.6 to 59.4% (Astal *et al*., 2005). Also, Kurutepe *et al.* (2005) found in a study that the resistance rate of *E. coli* to ciprofloxacin increased from 2.9% in 2000 to 11.3% in 2002. The results of the study emphasize the constant bacteriological evaluation and treatment line correction. Establishing a scientific supervision committee with standard definitions is highly recommended to supervise laboratory functions, the quality of the outputs and comparing them with referent laboratories. Using appropriate and standard disks is recommended. The irregular or improper use and prescription of drugs without any antibiogramic results except under certain circumstances have to be avoided in order to prevent causing and expanding resistance to new drugs with little observed resistance rates.

REFERENCES

Alos, J.I., M.G. Serrano, J.L. Gomez-Garces and J. Perianes, 2005. Antibiotic resistance of *Escherichia coli* from community-acquired urinary tract infections in relation to demographic and clinical data. Clin. Microbiol. Infect., 11: 199-203.

Andrade, S.S., H.S. Sader, R.N. Jones, A.S. Pereira, A.C. Pignatari and A.C. Gales, 2006. Increased resistance to first-line agents among bacterial pathogens isolated from urinary tract infections in Latin America: Time for local guidelines? Memórias Instituto Oswaldo Cruz, 101: 741-748.

Astal, Z.E., 2005. Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in the Gaza Strip. Singapore Med. J., 46: 457-459.

Bajaj, J.K., R.P. Karyakarte, J.D. Kulkarni and A.B. Deshmukh, 1999. Changing aetiology of urinary tract infections and emergence of drug resistance as a major problem. J. Commun. Dis., 31: 181-184.

Bartoloni, A., L. Pallecchi, M. Benedetti, C. Fernandez and Y. Vallejos *et al*., 2006. Multidrug resistant commensal *Escherichia coli* in children, Peru and Bolivia. Emerg. Infect. Dis., 12: 907-913.

Blomgran, R., L. Zheng and O. Stendahl, 2004. Uropathogenic *Escherichia coli* trigger oxygen-dependent apoptosis in human neutrophils through the cooperative effect of type 1 fimbriae and lipopolysaccharide. Infect. Immun., 72: 4570-4578.

Borji, A., S.S. Zahedani and A. Moradi, 2001. Survey resistance drug *E. coli* isolated of UTI in Zahedan. ZUMS J., 9: 28-32.

David, N. and M.B. Williams, 1996. Urinary tract infection. Post Graduate Med., 99: 189-191.

Fallah, F., H. Behzadnia, A. Moradi, G. Eslami, M. Sharifian, S. Rafiee and M.A. Malekan, 2008. Antimicrobial resistance pattern in urinary tract infections in children on continuous ambulatory peritoneal dialysis. Iran. J. Clin. Infect. Dis., 3: 155-159.

Foxman, B. and L. Riley, 2001. Molecular epidemiology: Focus on infection. Am. J. Epidemiol., 153: 1135-1141.

Ghotaslou, R., A.S. Oskouie and A. Mesri, 2005. The *in vitro* activity of ciprofloxacin on isolated organisms from urinary tract infections in a pediatric hospital. Pharm. Sci. Summer, pp: 29- 32.

Gupta, K., T.M. Hooten and W.E. Stamm, 2001. Increasing antimicrobial resistance and the management of uncomplicated community-acquried urinary tract infections. Ann. Intern. Med., 135: 41-50.

Hasan, A.S., D. Nair, J. Kaur, G. Baweja and M.P. Deb, 2007. Aggarwal, Resistance patterns of urinary isolates in a tertiary Indian hospital. J. Ayub Med. Coll. Abbottabad, 19: 39-41.

Inabo, H.I. and H.B.T. Obanibi, 2006. Antimicrobial susceptibility of some urinary tract clinical isolates to commonly used antibiotics. Afr. J. Biotechnol., 5: 487-489.

Jha, N. and S.K. Bapat, 2005. A study of sensitivity and resistance of pathogenic micro organisms causing UTI in Kathmandu valley. Kathmandu Univ. Med. J., 3: 123-129.

Kader, A.A., A. Kumar and S.M. Dass, 2004. Antimicrobial resistance patterns of gram-negative bacteria isolated from urine cultures at a general hospital. Saudi J. Kidney Dis. Trans., 15: 135-139.

Kresken, M. and D. Hafner, 2006. Further Increase of fluoroquinolone resistance among *Escherichia coli* isolates in a central European area. 16 ECCMID, Nice/France, April 1-4, http://www.blackwellpublishing.com/eccmid16/abstract.asp?id=50191.

Kurutepe, S., S. Surucuoglu, C. Sezgin, H. Gazi, G. Gulay and B. Ozckkaloglu, 2005. Increasing antimicrobial resistance in *Escherichia coli* isolates from community acquired urinary tract infections during 1998-2003 in Manisa, Turkey. Jap. J. Infect. Dis., 58: 159-161.

Laupland, K.B., S.M. Bagshaw, D.B. Gregson, A.W. Kirkpatrick, T. Ross and D.L. Church, 2005. Intensive care unit-acquired urinary tract infections in a regional critical care system. Crit. Care, 9: 60-65.

Mokhtarian, H., M. Ghahramani and H. Nourzad, 2006. A study of antibiotic resistance of *Escherichia coli* isolated from urinary tract infection. Ofogh Danesh Iran. J. Gonabad Univ. Med. Sci., 12: 5-11.

Nakhjavani, F.A., A. Mirsalehian, M. Hamidian, B. Kazemi, M. Mirafshar and F. Jabalameli, 2007. Antimicrobial susceptibility testing for *Escherichia coli* strains to fluoroquinolones, in urinary tract infections. Iran. J. Publ. Health, 36: 89-92.

Rebeecah, M., L. Carlton, R. Reid-Smith, C. Poppe and A. Scott *et al*., 2006. Chloramphenicol and kanamycin resistance among porcine *Escherichia coli* in Ontario. J. Antimicrobial. Chemother., 58: 173-177.

Selvarangan, R., P. Goluszko, J. Singhal, C. Carnoy, S. Moseley and B. Hudson, 2004. Interaction of Dr adhesion with collagen type IV is a critical step in *Escherichia coli* renal persistence. Infect. Immun., 72: 4827-4835.

Seputiene, V., M. Ruzauskas, P. Zlabys and E. Suziedeliene, 2006. Characterisation of streptomycin resistance determinants in Lithuanian *Escherichia coli* isolates. Biological, 2: 14-17.

Sydney, E.G.B., 1990. Selection Collection. In: Diagnostic Microbiology. Mosby, C.V. (Ed.). Behavioral Science Research, Australia, pp: 253-261.

Tamberkar, D.H., D.V. Dhanorkar, S.R. Gulhane, V.K. Khandelwal and M.N. Dudhane, 2006. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. Afr. J. Biotechnol., 5: 1562-1565.

Tankhiwale, S.S., S.V. Jalgaonkar, S. Ahamad and U. Hassani, 2004. Evaluation of extended spectrum âlactamase in Urinary isolates. Indian J. Med. Res., 120: 553-556.

Vaezzadeh, F. and M.K. Sharifi-Yazdi, 2001. Laboratory evaluation of urine culture and drug resistance in children clinically suspected of urinary tract infection. Iran. J. Publ. Health, 30: 123-124.

Zilevica, A., 2005. Hospital acquired and community-acquired uropathologens: Modeling of infection. Bioautomation, 3: 63-67.